Effect of ozonated water on the microbiological physical and nutritional quality parameters of minimally processed lettuce during shelf-life

Ölmez, H.[1], Leskinen, M.[b], Särkkä-Tirkkonen, M.[2]

Abstract

Since chlorine is not accepted for treatment of organic products, alternative sanitizing agents are needed to assure the safety and shelf-life of fresh-cut organic vegetables. Therefore, the effect of ozone on the microbiological, nutritional ands sensory quality of lettuce were studied during shelf-life at 4°C in this project. Ozone gas was produced from extra dry oxygen by means of a corona discharge generator. After spinning, samples were packaged using 35 µm oriented PP bags and stored at 4°C for 13 days. Sampling was done on days 0, 3, 8, 10, and 13. Analyses include aerobic plate count and psychrotrophic bacteria, vitamin C and sensory quality. One chlorine (100 ppm, 2 min) and one organic acid (0.25% citric acid plus 0.50% ascorbic acid, 2 min) disinfected sample were used as control samples during the shelf-life study, as chlorine is the generally used disinfecting agent in conventional processing and organic acids are one of the few alternatives that can be used for organic products. No significant difference was detected between the microbial quality of ozone, chlorine and organic acid disinfected lettuce samples. Although the vitamin C loss in 3 ppm ozone treated samples were significantly higher than the other samples, no significant difference was observed between chlorine washed and 1 ppm ozone treated samples. At the end of 10 days of storage, at a level of 5 %, no significant difference was observed between chlorine washed and the samples treated with 1 ppm ozone for 2 min.

Introduction

There has been an increasing demand for minimally processed products in recent years. Similar trends are valid also for organic products. Consumers now seek for processed organic products. Therefore, interest on the production of minimally processed organic vegetables has been also increasing. This results in a need for processing methods that enable minimally processed organic products with a comparable shelf-life and quality with their conventionally produced relatives. Due to the environmental and health risks that are posed by the use of chlorine (1,2), its use in organic production is forbidden in Europe. Moreover, in some European countries, use of chlorine has been forbidden even for conventional products. Thus, there is a need for alternative sanitizers to be used for the sanitization of fresh-cut vegetables not only for the organic food sector but also for the conventional food processors.

Organic acids have been investigated because of their bactericidal activity, and because they are generally recognized as safe (GRAS). However, antimicrobial activity changes very much among organic acids. Antimicrobial properties of acetic acid has been shown in inactivating *E.coli*, *L. monocytogenes*, *Salmonella* Typhimurium and *Yersinia enterocolitica*. Citric acid in the form of lemon juice has been demonstrated to reduce *S. Typhi* populations on fresh fruit. Common household sanitizers such as distilled water, apple cider vinegar (%5), lemon juice (%13), bleach

[1] TÜBİTAK Marmara Research Center, Food Institute, Kocaeli, Turkey
[2] University of Helsinki, Ruralia Institute, Mikkeli, Finland

(%4), and white vinegar (%35) decreased the numbers of aerobic bacteria by avarages of 0.6, 1.2, 1.8, 1.9, and 2.3 log/g, respectively, without causing severe effects in sensory quality of lettuce (3,4,5,6).

Previous studies on ozone proved that it has an antimicrobial activity higher than that of chlorine (7,8). It is a strong oxidant that can react with contaminants directly as molecular ozone or indirectly through the free radicals derived from it (9). Ozone acts more quickly than permissible levels of chlorine which makes it more suitable for washing procedures with short contact times. Moreover, since it does not pose a residue problem and does not create any by-products (7,8), it has been suggested as a good alternative for chlorine and have a potential to be used as a sanitizing agent for organically grown fresh cut vegetables.

Currently, ozone is accepted to be used in direct contact with foods in USA and in Europe for conventional products. But its use in organic products is not allowed in Europe yet. However, its important to note that in the IFOAM norms for organic production and processing, ozone is listed as a disinfectant to be used in direct contact with foods (10).

The previous studies on the use of ozone on lettuce were generally concentrated on the antimicrobial activity and did not include data on the effect of nutritional quality of the product. Only Beltran et al. (11) studied the effect of ozone treatment on the antioxidant constituents, besides other microbial and sensory quality parameters during the shelf-life of shredded iceberg lettuce. Therefore the aim of the current study was to assess the effect ozonated water washing on the microbiological, nutritional and physical quality parameters of minimally processed lettuce in comparison to aqueous chlorine and organic acid treatments during the shelf-life of the products.

Materials and Methods

Lettuce. Loose-leaf lettuce (*Lactuca sativa* L.) was purchased from a local producer. The core and the wrapper leaves were discarded. Leaves were shredded into about 3 cm pieces with a sterile knife. Then the leaves were first washed with tap water, and then with sterile deionized water. After the sanitizing treatment, a kitchen type vegetable spinner was used for spinning to remove excess water on the lettuce leaves.

Washing solutions and procedure for treating lettuce. All the glassware used were washed and rinsed with deionized water, autoclaved and then dried to obtain ozone and chlorine demand-free glass-ware. Ozonated water was prepared by ozonating sterile distilled water. Gaseous ozone was generated using a corona discharge ozone generator (model Micronix, Mikron Makina Ltd., Istanbul, Turkey) from purified, extra-dry oxygen gas. The ozone generator has a capacity of 15 g ozone h^{-1}. Water was ozonated by using a Mini Ozone Injection System (Ozone Solutions, Inc., Sioux Center, IA, USA) equipped with a pump, a contact tank having a water inlet tube, pressure gauges and regulators, safety release valve, liquid withdrawal tube and gas inlet tube fitted with a venturi-type injection dispenser unit. Ozone was introduced to the water by means of the injection system until the required ozone concentration was attained. Sodium hypochlorite solution (100 ppm free chlorine) and 0.25 % citric acid plus 0.50 % ascorbic acid solution were also prepared using sterile distilled water. Ozone and chlorine concentrations were measured by colorimetrically (Spectroquant Colorimeter Picco, Merck, Darmstadt, Germany).

Microbial analysis. Total aerobic plate count, *Enterobactericeae*, and psychrotrophic bacteria were enumerated immediately after the sanitizing treatment and at regular intervals during the shelf-life. Total aerobic plate count was determined on plate count agar (PCA, Oxoid, Basingstoke, England) following the pour plate method by incubation at 35 °C for 48 h. Psychrotrophic bacteria were determined on plate count agar (PCA, Oxoid, Basingstoke, England) following the pour plate method by incubation at 4°C for 10 days. The number of *Enterobacteriaceae* was determined on Violet Red Bile Glucose Agar (VRBGA, BioRad, Marnes la Coquette, France) using the pour plate method. The results of the microbiological analyses were expressed as the logarithm of colony-forming unit per gram of fresh weight of lettuce (log cfu/g lettuce).

Analysis of vitamin C. Vitamin C was analyzed according to the modified indophenol titrimetric method as described in AOAC (1995). The results were expressed as mg/100 g of fresh lettuce.

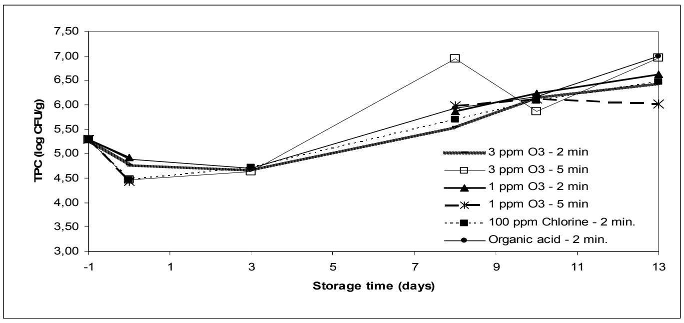
Shelf-life study. Samples were packaged using 35 μm oriented PP bags and stored at 4ºC for 13 days. Sampling was done on days 0, 3, 8, 10, 13. Analyses include aerobic plate count, *Enterobacteria*, vitamin C, colour and sensory analysis. One chlorine (100 ppm) and one organic acid (0.25% citric acid plus 0.50% ascorbic acid) disinfected sample were used as control samples during the shelf-life study.

Statistical analysis. All the given results were obtained from duplicated samples, and each point was replicated twice. Analysis of variance (ANOVA) was performed followed by post hoc Tukey's test with a level of significance at $p < 0.05$ using STATISTICA.

Results and discussion

Effect of Sanitizing Treatment on Microbial Quality

The results of microbial analysis were demonstrated in Figure 1. No significant difference was observed between the sanitizing treatments, in terms of change in the microbial load of the product during the shelf-life at 4°C. Even at the end of the 13th day of storage, the microbial load was lower than 10^7 CFU/g. The ozone treatment was found to be as effective as chlorine wash in terms of microbial inactivation.



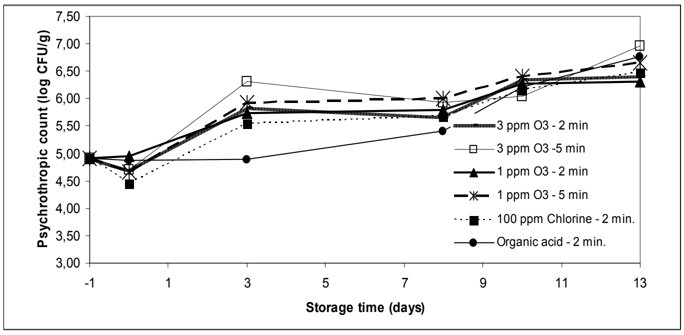


Figure 1. Changes in microbial load of lettuce lettuce washed with ozone compared to that treated with chlorine (100 ppm) and organic acid (0.25 % citric acid + 0.50 % ascorbic acid) during the shelf-life 4ºC for 13 days.

3 ppm ozone treatment resulted in a significantly ($p<0.05$) higher reduction in Vitamin C content compared to 1 ppm ozone and 100 ppm chlorine treatments (Figure 2.). No significant ($p\geq 0.05$) difference was observed between the Vitamin C contents of 1 ppm ozone and 100 ppm chlorine treated samples.

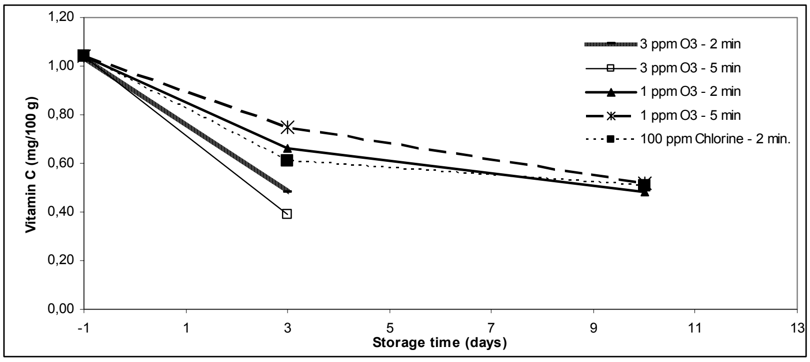


Figure 2. Changes in Vitamin C content of lettuce lettuce washed with ozone compared to that treated with chlorine (100 ppm) during the shelf-life 4ºC for 13 days.

Sensory analyses were done by 8 trained panellists, in which the samples were ranked according to overall appearance, odour, and taste. Samples were taken at days 5 and 10. Since a high discoloration and tissue softening occurred in the samples treated with 3 ppm ozone, they were discarded after 2-4 days of storage and were not included in the sensory analysis. Moreover, all the samples were discarded at day 13, since unacceptable browning and tissue softening developed. No significant difference (at a level of 5 %) was observed between the rest of the samples in terms of appearance and odor, whereas taste of the 1 ppm O_3 – 2 min sample was found to be superior at day 5. The appearance of chlorine washed sample was significantly better than the others at day 10. Moreover, the organic acid treated and the 1 ppm O_3 – 5 min. samples were ranked to be the lower than the other two sample in all aspects at day 10.

In terms of retarding microbial growth and slowing down organoleptic changes, washing with 1 ppm ozone for 2 min at 10°C was found to be a good alternative for chlorine disinfection of minimally processed lettuce.

Acknowledgement

The authors gratefully acknowledge funding from the European Community financial participation under the Sixth Framework Programme for Research, Technological Development and Demonstration Activities, for the Integrated Project QUALITYLOWINPUTFOOD, FP6-FOOD-CT-2003- 506358.

References

1. Korichi, D. G., J. R. Mead, M. S. Madore, N. A. Sinclair, and C. R. Sterling. 1990. Effects of ozone, chlorine dioxide, chlorine, and monochloramine on *Cryptosporidium parvum* oocyst viability. *Appl. Environ. Microbiol*. 56:1423-1428.
2. Wei, C. I., D. L. Cook, and J. R. Kirk. 1985. Use of chlorine compounds in the food industry. *Food Technol*. 39:107-115.
3. Fernandez Escartin, E.F., Castillo Ayala, A. and Saldana Lozano, J. 1989. Survival and growth of *Salmonella* and *Shigella* on sliced fresh fruit. *J Food Prot* **52:** 471-472.
4. Torriani, S., Orsl, C. and Vescovo, M. 1997. Potential of *Lactobacillus casei*, culture permeate, and lactic acid to control microorganisms in ready-to-use vegetables. *J Food Prot* **60:**1564-1567.
5. Adams, M.R., Hartley, A.D. and Cox, L.J. 1989. Factors affecting the efficacy of washing procedures used in the production of prepared salads. *Food Microbiol* **6:** 69-77.
6. Zhang, S. and Farber, J.M. 1996. The effects of various disinfectants against *Listeria monocytogenes* on fresh cut vegetables. *Food Microbiol* **13:** 311-321.
7. Kim, J. G., A. E. Yousef, and G. W. Chism. 1999. Use of ozone to inactivate microorganisms on lettuce. *J. Food Safety* 19:17-34.
8. Achen, M. and A. E. Yousef. 2001. Efficiacy of ozone against *Escherichia coli* O157:H7 on apples. *J. Food Sci.* 66:1380-1384.
9. Koseki, S., K. Yoshida, S. Isobe, and K. Itoh. 2001. Decontamination of lettuce using acid electrolyzed water. *J. Food Prot*. 64:652-658.
10. IFOAM 2005. The IFOAM Norms for Organic Production and Processing. Version 2005, Germany.
11. Beltran, D., M. V. Selma, A. Marin, and M. I. Gil. 2005. Ozonated water extends the shelf life of fresh-cut lettuce. *J. Agric. Food Chem.* 53:5654-5663.
12. AOAC, 1995. AOAC Offical Method 985.33 Vitamin C in Ready-to-Feed Milk-Based Infant Formula, 2,6-Dichloroindophenol Titrimetric Method. Official Methods of Analysis. 16th. Edition, Association of Official Analytical Chemists. Washington, DC., USA.